Robust Summary - Group 2: Low Butadiene C4

Acute Toxicity

Test Substance	Isobutylene 99.4% pure. Purity determined by Nat'l Bureau of Standards freezing point method.
Method	
Method/guideline followed	No guideline specified, acceptable scientific method
Type (test type)	Acute effects evaluation
GLP	No
Year	1950
Species/Strain	dog, strain (or breed) not specified
Sex	Not specified
No. of animals per sex/dose	4 dogs
Vehicle	air
Route of administration	Inhalation
Test Conditions	This pharmacology study was performed to elucidate relationships between chemical structure and physiological activity. Of particular interest was the ratio of anesthetic to respiratory arrest concentrations (anesthetic index) in the mouse and the specific characteristic of inducing severe arrhythmia/fibrillation in surgically anesthesized dogs after IV injection of epinephrine (method of Meeks et al., 1937 and Carr & Krantz, 1949). Dogs (age not reported) were administered each of the 9 test materials including isobutylene, 1- butene or 2-butene, cis, at sufficient dose and duration to induce an appropriate level of anesthesia followed by I.V. administration of epinephrine (exact dose not reported) to produce cardiac stimulation. Reviewer comments: Compounds that can sensitize the heart in this test are believed to be ones that might induce heart irregularities under stressful conditions.
Results	
LC_{50} with confidence limits. Remarks	No LC 50 was determined. Arrhythmias of different levels of severity were produced with each agent. The arrhythymias were least severe with isobutylene, which produced only mild tachycardia and minor voltage changes after epinephrine injection in all 4 dogs, suggesting a wider margin of safety in exposure conditions.
Conclusions (study author)	Irregularities of cardiac rhythm of at least moderate severity were produced with all compounds except isobutylene that caused only mild tachycardia and minor voltage changes after epinephrine injection.
Data Quality Reliability	**2**. Reliable with restrictions. This is not a standard acute toxicity study. It is a research study using non-standard methods that were appropriate for the purpose.
References	Virtue, R.W. 1950. Anesthetic Effects in Mice and Dogs of Some Unsaturated Hydrocarbons and Carbon Oxygen Ring Compounds. Pro. Soc. Exp. Biol. Med. 73: 259-262 (See additional acute summary on mouse research) Meek, W.J., Hathaway, H.R. and Orth, O.S. 1937. J. Pharm. Exp. Thera. 61: 240. Carr, C.J. and Krantz, J.C. 1949. Fed. Proc. 8:279.
Other	
Last changed	Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Acute Toxicity

Test Substance	Isobutylene 99.4% pure. Purity determined by Nat'l Bureau of Standards freezing point method.
Method	
Method/guideline followed	No guideline specified, acceptable scientific method
Type (test type)	Acute Effects Evaluation
GLP	No
Year	1950
Species/Strain	Mouse, strain not reported
Sex	not specified
No. of animals per sex per dose	approx 64 mice used to obtain each reported value
Vehicle	oxygen
Route of administration	whole body inhalation
Test Conditions	The purpose of this research study was to compare the anesthetic properties of 20 different highly purified unsaturated hydrocarbons and carbon-oxygen ring compounds in mice (sex/age not reported). Concentrations required for surgical anesthesia and for respiratory arrest were measured. Experiments were carried out in a large stoppered jar equipped with apparatus to introduce known quantities of oxygen (21%) or test compound at 25-27^{0}C under atmospheric pressure. CO2 was absorbed with NaOH. Experiments were limited to concentrations causing anesthesia in 10 min. and were terminated after 20 min. Probit analysis was used to determine conc/effect relationships.
Results	
LC_{50} with confidence limits.	LC50s were not measured. For isobutylene, surgical anesthesia occurred at a concentration of 19.8% and respiratory arrest at 32% giving an anesthetic index of 1.6.
Remarks	Isobutylene demonstrated the widest range between anesthesia and respiratory arrest in this series, suggesting a better margin of safety.
Conclusions (study author)	Results support the concept that narcotic potency increases with molecular weight and degree of unsaturation.
Data Quality Reliability	2. Reliable with restrictions. This is not a standard acute toxicity study. It is research study using non-standard methods. Mouse strain and sex were not specified. Methods were appropriate for the purpose.
References	Virtue, R.W. 1950. Anesthetic Effects in Mice and Dogs of Some Unsaturated Hydrocarbons and Carbon Oxygen Ring Compounds. Proc. Soc. Exp. Biol. Med 73:259-262. (See additional acute summaries on other mouse and dog research)
Other	
Last changed	Rev.6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Isobutylene, 99.8% liquefied.
Method	
Method/guideline followed	Comparable to standard bacterial mutation assays
Type	Reverse mutation bacterial
System of testing	Ames Salmonella assay with and without metabolic activation and E. coli
GLP	GLP
Year	1981
Species/Strain	S. typhimurium TA1535, TA1537, TA1538, TA100, TA98; E. coli WP2uvrA(pKM101)
Metabolic activation	Yes
Species and cell type	Male rat liver
Quantity	50µl S-9 homogenate in 0.5ml S-9mix/plate
Induced or not induced	Aroclor 1254 induced – 500 mg/kg in corn oil, administered 5 days prior to sacrifice.
Concentrations tested	1st test: 5, 10, 20, 30, 40, 50%. 2nd test: 10, 20, 40, 60, 80, 100%
Statistical Methods	None employed. Criteria for positive responses were, for TA100 a 1.5 fold increase and for TA1535, TA1537, TA1538, TA98 and E.coli, a doubling of revertant colonies compared to mean negative control values at any dose. Tests were also observed for dose response.
Remarks for Test Conditions	Bacteria were freshly prepared by 16 hour culturing in nutrient broth prior to use and monitored for strain sensitivity. An agar overlay comprised of 2 ml agar, 0.5ml S-9 mix or phosphate buffer, and 0.1ml fresh bacteria was mixed and poured on minimal agar plates. When set, plates were inverted, placed in jars of known volume and exposed to isobutylene at 37^0C for 48 hours, then incubated an additional 24 hours in fresh air. Concentrations of isobutylene were achieved by mixing hydrocarbon-free artificial air and test gas through flow meters before delivery into incubation jars. Flow meters were calibrated by comparing standard registered flow rates with actual flow rates measured by gas burette at atmospheric pressure and ambient temp. Actual flow rates were obtained by multiplying registered air flow rates by the appropriate conversion factor. Approx. 25 liters gas/air filled each 6.25 liter jar during exposure. Actual gas concentrations inside the incubation jars were not measured. Duplicate plates were used in the first trial for each test, only one plate was used at each dose in the repeat trial/test. Negative control: hydrocarbon free artificial air, Positive gas control: vinyl chloride 30% in air in TA 1535, TA100 ± S9, Other pos. controls: 4-actyl aminofluorene 1.0mg/plate in TA1538, TA98 +S9; methyl methane sulfonate 100 µg/plate in E.coli –S9, and 9-amino acridine 20µg/plate in TA1537 -S9.
Results	
Genotoxic effects	No mutagenic activity was induced by isobutylene in any strain at any concentration in the first or second tests. Reduction in number of colonies in all strains indicative of toxicity and growth inhibition was observed with and without metabolic activation at 80% and 100% isobutylene. Positive controls responded appropriately, inducing from 3 fold –30 fold increases above negative controls ±S9.
Conclusions	
(contractor)	Isobutylene was adequately tested at sufficiently high doses to induce toxicity, and is not mutagenic to bacteria in this test system.

Data Quality *Reliabilities*	**2.** Reliable with restrictions. Only 2 plates/dose in initial trial and only 1 plate/dose in repeat trial of each test was used. Gas concentration within chambers was not measured.
Reference	McGregor, D.B., Reach,C.G. 1981. Isobutylene: Ames test for Mutagenic Activity with Salmonella TA 1535, TA100, TA1537, TA1538, TA98, and E.coli WP2 uvrB)pKM101), unpublished Rpt# 2098, IRI Proj. 704338 Inveresk Research Institute, for Essochem Europe, Inc. Machelen, Belgium
Other *Last changed*	Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel).

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Isobutylene, liquefied, from Essochem Europe, Inc. CAS Number 115-11-7.
Method	
Method/guideline followed	Adequate scientific method based on Clive et al (1972, 77, 79), Amacher et al (1979)
Type	Mammalian cell point mutation assay
System of testing	Mouse lymphoma
GLP	Yes
Year	1981
Species/Strain	Mouse lymphoma L5178Y TK^+/TK^- cell line from Clive
Metabolic activation	Yes
Species and cell type	Male Fischer 344 rat liver
Quantity	1 ml S-9/flask (9 parts cofactors:1 part 9000 G liver prep)
Induced or not induced	Aroclor 1254 induced. Administered ip 500 mg/kg, 5 days prior to sacrifice
Concentrations tested	100% or 50, 25, 12.5 , 6.25% isobutylene diluted with 5% CO_2 in air
Statistical Methods	None employed. Positive response is defined as a doubling of mutant frequency (mutant colonies ÷ 10^5 survivors) compared to solvent controls with a dose response over two consecutive concentrations. An increase in absolute mutant colonies is highly desirable.
Remarks for Test Conditions	In the preliminary toxicity test, mouse lymphoma cells ($3x10^6$ cells) in culture flasks were exposed to isobutylene at concentrations of 100 – 6.25% without metabolic activation in incubation jars. Concentrations were blended by passing air and isobutylene through flow meters into a mixing chamber, before delivery into the incubation jars. Flow meters were calibrated by comparing standard registered flow rates with actual flow rates measured by gas burette at atmospheric pressure and ambient temperature. Actual flow rates were obtained by multiplying registered flow rates by appropriate conversion factor. Approximately 25 l gas/air mixture was flushed through each 6.25 l jar during exposure. Actual gas concentrations in jars were not measured. Incubation was carried out with shaking for 24 hours at 37^0 C. After incubation, test atmosphere was removed and cells were harvested by centrifugation. Resuspended cells were transferred to fresh tissue culture flasks, gassed with 5% CO_2 in air and incubated at 37^0 C. Cell density was measured each day for three days by counting with a Neubauer haemocytometer to determine toxicity. In the definitive mutation test, 10 ml of $3x10^6$ exponentially growing L5178Y cells were exposed to isobutylene at concentrations of 100%-6.25% with and without metabolic activation. All cultures were incubated with shaking (150 rpm) at 37^0 C for 24 hours. Positive control compund without S-9 was ethyl methane sulfonate (400, 200 µg/ml); with S-9, 2-acetylamino fluorine (100, 50 µg/ml); cultures were treated for 3 hours. After incubation, cells were harvested by centrifugation, resuspended in fresh medium, and samples from each suspension plated on soft agar for varying times. For day 0 survival, cells were plated immediately after exposure (3 plates/dose level), allowed to set at 4^0 C, equilibrated with 5% CO_2/air and incubated at 37^0 C for 10 days. For expression of genetic damage, cells multiplied in liquid medium for 3 days following exposure. On the third day, cell cultures were adjusted to $3x10^5$ cells/ml, diluted in cloning medium, dispensed to 3 plates /dose level and incubated at 37^0 C for 10 days to determine cell survival. For mutant colony selection, cells were dispensed into cloning medium containing 5 µg/ml

	triflurothymidine (TFT), 3 plates/ dose group, and incubated at 37^0 C for 7-10 days. At the end of incubation, mutant colonies were counted manually.
Results Genotoxic effects	Preliminary toxicity results in the absence of S-9 indicated severe toxicity at 100% isobutylene due either to isobutylene itself or prolonged hypoxia to cells caused by exposure to 100% test gas atmosphere. Varying degrees of toxicity also occurred at other doses, only the lowest dose 6.25% was non-toxic. In the first of two mutation tests without S-9, cultures treated with isobutylene induced more TFT resistant colonies than controls but no mutant frequencies reach doubling. Numbers of colonies on survival plates were lower than normal producing overall higher mutant frequencies. These unusual distributions were due to inadequate precleansing of cultures with methotrexate prior to use. In the second experiment, following two additional rounds of cleansing with methotrexate, the number of mutant colonies induced by isobutylene and those in the negative control cultures were much lower and the number of survival colonies much increased. No dose of isobutylene induced a mutant frequency greater than the negative control. Of three experiments performed with S-9, the first was rejected because incubation with S-9 for 24 hours killed 80-90% cells in all cultures including the positive controls, and inadequate cleansing with methotrexate resulted in excess mutant colonies in the negative control group. In the subsequent 2 tests, shorter exposure of 16 hours substantially reduced S-9 induced toxicity. Exposure to isobutylene at concentrations up to 100% did not result in any significant increase in mutant colonies compared to negative control (CO2/air) cultures. Positive control treatment produced appropriate increases in mutant frequency.
Conclusions (contractor)	In both the absence and presence of S-9 mix, isobutylene showed no evidence of mutagenic activity in the mouse lymphoma assay.
Data Quality *Reliabilities*	**2**-Reliable with restrictions. No direct measurement of exposure concentration or analysis of incubation jar atmosphere was performed. Results of these tests are valid and the lack of mutagenic effect was reproducible despite poor initial cell cleansing and toxicity due to initial overexposure to S-9.
References	McGregor, D.B., Ross, C.A. 1981. Isobutylene: Assessment of mutagenic potential in the Mouse lymphoma mutation assay. Inveresk Research International, Musselburgh, Scotland for Essochem Europe Inc., Machelen, Belgium. Clive et al. 1972. Mut. Res. 77-87; 1977 Handbook of Mutagenicity Test Procedures, Kilbey et al. Eds., Elsevier, pp161-173; 1979 Mut. Res. 59: 61-108. Amacher et al., 1979. Mut. Res. 64: 391-406.
Other *Last changed*	Revised 5/17/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Isobutylene, liquefied, from Essochem Europe, Inc. CAS Number 115-11-7
Method	
Method/guideline followed	Adequate scientific method based on Heidelberger
Type	In vitro Cell Transformation
System of testing	Mouse embryo fibroblast derived cell line
GLP	Yes
Year	1981
Species/Strain	C3H/10T½ Cl 8 mouse cell line
Metabolic activation	Yes
Species and cell type	Male Fischer 344 Rat liver
Quantity	5% S-9 mix (9 parts cofactor:1 part 9000 G liver prep/flask)
Induced or not induced	Aroclor induced: 500 mg/kg administered ip 5 days prior to sacrifice
Concentrations tested	Prelim. Tox: 100% isobutylene or 50, 25, 12.5 ,6.25% diluted with 5%CO_2 in air Transformation assay: 100%, 50, 25% in 5% CO_2/air
Statistical Methods	None employed. Positive response is defined as the presence of type II or type III transformed foci in treated cultures with evidence of dose response and reproducibility in repeat assay. Compounds which transform fibroblast cells have a high probability of inducing tumors if injected in immunosuppressed mice.
Remarks for Test Conditions	Preliminary toxicity assay without metabolic activation was performed to establish a range of concentrations for the transformation assay. Five ml. Samples of cells from a culture at density of 200 cells/ml were pipetted into plastic tissue culture flasks, incubated in 5% CO_2/air overnight for equilibration, then medium was replaced with fresh medium supplemented with fetal bovine serum (10% v/v). Flasks with caps screwed on lightly were placed in incubation jars which were flushed with 100% isobutylene or isobutylene mixed with 5% CO_2/air to achieve concentrations ranging from 50%-6.25%. Concentrations were blended by passing air and isobutylene through flow meters into a mixing chamber, before delivery into the incubation jars. Flow meters were calibrated by comparing standard registered flow rates with actual flow rates measured by gas burette at atmospheric pressure and ambient temperature. Actual flow rates were obtained by multiplying registered flow rates by appropriate conversion factor. Approximately 25 l gas/air mixture was flushed through each 6.25 l jar during exposure. Actual gas concentrations in jars were not measured. Jars were sealed and incubated with shaking (50 rpm) at 37^0 C for 24 hours. Exposure medium was then replaced with fresh medium and culture flasks incubated for an additional 3 weeks. Cells were harvested with trypsin and counted for toxicity in Neubauer haemocytometers. For the transformation assay, cultures were treated as above, except that S-9 mix was added to one half flasks (6/dose group) and all flasks (12/dose group) were placed in incubation jars flushed with 100%, 50% or 25% isobutylene. After 24 hours incubation with shaking, medium was changed and cells were incubated in flasks for 8 weeks. Medium was changed twice weekly until cells reached confluence and weekly thereafter. At 8 weeks, cells were fixed in methanol, stained with Giemsa and scored for transformed foci. Positive control chemicals were 3-methylcholanthrene (30, 15 µg/ml), ethyl methane sulfonate (250, 125 µg/ml), 2-acetylaminofluorene (10, 5 µg/ml) and 2-aminoanthracene (5, 2.5 µg/ml). Negative controls were CO_2/air, DMSO or acetone.

Results Genotoxic effects	In the preliminary toxicity test without S9, only 100% isobutylene caused cell toxicity either due to isobutylene itself or prolonged hypoxia resulting from exposure to 100% test gas atmosphere. In the transformation assay with or without metabolic activation, no transformed colonies were observed at any exposure level. Positive control compounds, known carcinogens in vivo, induced clear evidence of morphological transformation.
Conclusions (contractor)	By criterion used in this laboratory, isobutylene had no transforming effect in C3H/10T½ cells in the presence or absence of liver metabolic activation and is not considered a potential carcinogen in vivo.
Data Quality *Reliabilities*	**2-**Reliable with restrictions. No direct measurement of exposure concentration or analysis of incubation jar atmosphere was performed
Reference	McGregor, D.B., Poole, A. 1981. Isobutylene: Induction of morphological transformation in C3H/10T½ clone 8 cells. Inveresk Research International, Musselburgh, Scotland for Essochem Europe, Inc., Machelen, Belgium
Other *Last changed*	Revised 5/17/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vivo

Test Substance	
Remarks	Isobutylene, colorless gas, 100% pure.
Method	
Method/guideline followed	Consistent with standard methods. Cites Heddle et al. 1983 Report of US EPA GeneTox Program Mut. Res. 123: 61-119 and Cunningham et al.1986 Mutagenesis 1: 449-452.
Type	Mammalian Bone Marrow Erythrocyte Micronucleus Test
GLP	Yes
Year	1990
Species	Mouse
Strain	B6C3F1
Sex	50 males (10/group)
Route of administration	Whole body Inhalation
Doses/concentration levels	1000, 3260, 10,000 ppm in air; Positive control 1,3-butadiene (1000 ppm)
Exposure period	6 hours/day for 2 days
Statistical methods	Calculation of mean and std. dev. of micronuclei data. Test of equality of group means by standard ANOVA at each time period, followed by Duncan's Multiple Range test if ANOVA was significant. Standard regression used for dose response. Residuals of ANOVA analyzed for normality by Wilk's Criterion.
Remarks for Test Conditions.	Male mice (10/group) were exposed to isobutylene, 6 hours a day for two days at 0, 1000, 3260 or 10,000 ppm. Actual exposure concentrations were determined by on-line gas chromatography reported hourly. Nominal concentrations were calculated. Chamber homogeneity verified by GC in pretrials. All mice were killed 24 hours after second exposure. Bone marrow was removed from both femurs, slides were prepared and stained with acridine orange for fluorescence. 1000 polychromatic erythrocytes (PCEs) were examined for micronuclei. Ratio of PCEs to normochromatic erythrocytes (NCEs) was determined by counting 1000 erythrocytes (PCE + NCE).
Results	
Genotoxic effects	
NOAEL (NOEL)	NOAEL = 10,000 ppm
LOAEL (LOEL)	Isobutylene did not induce a statistically significant positive response nor a dose-related increase in the number of micronuclei in PCEs of mouse bone marrow at any dose level. A significant regression coefficient ($p < 0.05$) for increased percentage of PCEs was observed. This event was within historical control values and is not considered biologically significant. PCE/NCE ratios were unremarkable, averaging 56-60% in treated males and 56-58% in treated females compared to negative controls (57%M, 55%F). Positive control 1,3-butadiene induced statistically significant increases in micronuclei and a reduced %PCE indicative of toxicity. Negative control values were within normal range.
Conclusions	
(study authors)	Isobutylene was not clastogenic in mouse bone marrow under conditions of this test system.
Data Quality	
Reliabilities	**1**. Reliable without restriction
References	Przygoda, R. 1990. In vivo mammalian bone marrow micronucleus assay for isobutylene. Project #236030. Exxon Biomedical Sciences Inc. East Millstone, NJ
Other	
Last changed	Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	
Remarks	Isobutylene, 99.7% pure, provided by study sponsor.
Method	
Method/guideline followed	No guidelines specified, acceptable scientific method
Test type	Subacute toxicity
GLP	Yes
Year	1986
Species	rat
Strain	Sprague Dawley, CD(SR)BR
Route of administration	Oral gavage
Duration of test	4 weeks
Doses/concentration levels	0, 1.49, 14.86, 148.55 mg/kg/day (nominal doses). Test article preparations were considered acceptable with analytical characterizations in the range of 71-134% of nominal conc. Doses were selected based on range-finding study #4298-13-20.
Sex	5M, 5F/group
Exposure period	4 weeks
Frequency of treatment	once/day, 7 days/week
Control group and treatment	5M, 5F; corn oil vehicle
Post exposure observation period	none
Statistical methods	Not specified. Group means and std. dev. calculated.
Test Conditions	Groups of rats (5M,5F/group, approx. 42 days old at start) received a daily oral dose (5ml/kg) of corn oil containing various levels of isobutylene, 7 days a week for 4 weeks. Pelleted diet and tap water were available ad lib. Rats were examined twice daily for morbidity and mortality. Body weights were recorded weekly. Blood for hematology and clinical chemistry was collected during week 4. At sacrifice, necropsies were performed and tissues preserved on all rats. Histopathologic evaluations were performed on tissues from all rats in group 1(corn oil control) and group 4 (High dose)
Results	
NOAEL (NOEL)	NOEL = 14.86 mg/kg/day
LOAEL (LOEL)	LOEL = 148.55 mg/kg/day
Remarks	The only statistically significant treatment related effects were a decrease in total white blood cell count of 11% (M, p<0.01) and 44% (F, p<0.01) in group 4 rats, predominantly in leucocytes and monocytes. Differential counts of WBC cell types were performed but not analyzed statistically. Slight, non-significant increases in BUN (M) and blood glucose (F) in group 4 were also observed. The range finding study (#4298-13/19-20) showed very low levels of isobutylene in blood after dosing with 29.7 mg/kg (nominal) reaching a maximum of 1.2 µg/ml 20 min after dosing, and a maximum of 17% of the dose in the GI tract 20 min after dosing
Conclusions	
(study authors)	No toxicologically significant changes were observed at dose levels up to 148.6 mg/kg/day administered over 4 weeks. Reviewer comments: A reasonable explanation for the low recovery of isobutylene might be that a considerable amount was lost back to the atmosphere via volatilization after instillation as a bolus dose in the warm stomach.

Quality Reliabilities	 **2**. Reliable with restrictions. Statistical method used was not reported
References	Jones, R.P. 1986. Isobutylene: 4 week oral (gavage) toxicity study in the rat, # 4372-13/21, Hazleton Laboratories Europe Ltd. for Essochem Europe Inc, Machelen, Belgium Jones, R.P. 1986. Isobutylene: Effects of single and repeated oral dosing in the rat (Range finding study) #4298-13/19-20, Hazleton Laboratories Europe Ltd. 1- Isobutylene –preparation and analysis of corn oil formulations: a feasibility study. 1985. #4188-13/7, Hazleton Laboratories Europe Ltd.
Other Last changed	 Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Repeated Dose Toxicity

Test Substance	
Remarks	Isobutylene, 99.7% pure, provided by study sponsor, CAS Number 115-11-7
Method	
Method/guideline followed	No guidelines specified, acceptable scientific method
Test type	Inhalation Subchronic
GLP	Yes
Year	1982
Species	rat
Strain	Sprague Dawley Crl:CD(SR)BR
Route of administration	Whole body inhalation
Duration of test	13 weeks
Doses/concentration levels	0, 250, 1000, 8000 ppm
Sex	10M, 10F/group
Exposure period	13 weeks
Frequency of treatment	6 hours/day, 5 days/week
Control group and treatment	10 M, 10 F; filtered room air exposed
Post exposure observation period	not applicable
Statistical methods	Analysis performed for the following parameters: body weight, body weight gain, hematology, blood chemistry, organ weights, organ/body wt ratio, organ/brain wt. ratio. Analysis of variance used for normally distributed errors, t-test between control and treatment groups. For non-normal distributions, Kruskal-Wallis test was used; significance determined by the Wilcoxan rank sum test. All tests were two tailed
Test Conditions	Groups of rat (10 M,10 F/group, approx. 47 days old at start) were exposed to isobutylene at 0, 250, 1000, 8000 ppm 6 hrs/day, 5 d/week for 13 wks. Water and pelleted diet were available ad lib. Rats were observed twice daily for morbidity and mortality. Body weight and food consumption were recorded weekly. Fasted blood was collected at initiation, wk 5, and wk 13 for hematology and chemistry. Urine samples were obtained during wk 13 for chemistry. At sacrifice bone marrow was collected, opthalmoscopy and necropsies were performed, and tissues preserved for histopathology.
Results	
NOAEL (NOEL)	NOEL = 8000 ppm
LOAEL (LOEL)	LOEL not determined
Remarks	No biologically significant treatment related effects were observed at any dose level. In the intermediate and high dose males and females, elevated ketones were detected in urine (Multistix, semi-quantitative method).
Conclusions (study authors)	No biologically significant treatment related effects were found. The 8000 ppm dose level was the highest that could be tested while ensuring that the chamber concentration would be below the lower explosive limit of isobutylene. Reviewer comments: Toxicological significance of elevated ketones is unknown but the finding indicates absorption of the test article. Possibly urine ketone bodies were derived from metabolism of the 4-carbon isobutylene. It was likely that internal organ exposure was higher in this inhalation study that in the oral studies where ketone bodies were not found (#4298-13/19-20). However, blood and organ levels were not measured after inhalation.

Quality Reliabilities	**1.** Reliable without restriction
References	Blackett, N.T. 1982. Isobutylene: 13 week inhalation toxicity study in the rat, # 2916-13/11, Hazleton Laboratories Europe Ltd. for Essochem Europe Inc, Machelen, Belgium Jones, R.P. 1986. Isobutylene: Effects of single and repeated oral dosing in the rat (Range finding study) #4298-13/19-20, Hazleton Laboratories Europe Ltd. For Essochem Europe Inc., Machelen, Belgium
Other Last changed	Revised 5/17/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Acute Toxicity

Test Substance	1-butene 99.88% pure. Purity determined by Nat'l Bureau of Standards freezing point method.
Method	
Method/guideline followed	No guideline specified, acceptable scientific method
Type (test type)	Acute Effects Evaluation
GLP	No
Year	1950
Species/Strain	Mouse, strain not reported
Sex	not specified
No. of animals per sex per dose	approx 64 mice used to obtain each reported value
Vehicle	oxygen
Route of administration	whole body inhalation
Test Conditions	The purpose of this research study was to compare the anesthetic properties of 20 different highly purified unsaturated hydrocarbons and carbon-oxygen ring compounds in mice (sex/age not reported). Concentrations required for surgical anesthesia and for respiratory arrest were measured. Experiments were carried out in a large stoppered jar equipped with apparatus to introduce known quantities of oxygen (21%) or test compound at 25-27^{0}C under atmospheric pressure. CO2 was absorbed with NaOH. Experiments were limited to concentrations causing anesthesia in 10 min. and were terminated after 20 min. Probit analysis was used to determine conc/effect relationships.
Results	
LC_{50} with confidence limits.	LC50s were not measured. For 1-butene, surgical anesthesia occurred at 22.7%, and respiratory arrest at 27.2% giving an anesthetic index of 1.2.
Remarks	
Conclusions (study author)	Results support the concept that narcotic potency increases with molecular weight and degree of unsaturation.
Data Quality Reliability	**2**. Reliable with restrictions. This is not a standard acute toxicity study. It is research study using non-standard methods. Mouse strain and sex were not specified. Methods were appropriate for the purpose.
References	Virtue, R.W. 1950. Anesthetic Effects in Mice and Dogs of Some Unsaturated Hydrocarbons and Carbon Oxygen Ring Compounds. Proc. Soc. Exp. Biol. Med 73:259-262. (See additional acute summaries on other mouse and dog research)
Other	
Last changed	Rev.6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vitro

Test Substance	
Test substance	1-butene, highest purity from Matheson Scientific.
Method	
Method/guideline followed	New method validation to evaluate model vapor-phase chemicals for mutagenicity either in solution or by an adsorption/desorption technique.
Type	Reverse mutation bacterial
System of testing	Ames Salmonella assay with or without metabolic activation
GLP	No
Year	1984
Species/Strain	Salmonella typhimurium TA97, TA98, TA 100
Metabolic activation	Yes
Species and cell type	Male Sprague Dawley rats or Syrian Golden hamsters
Quantity	500 µl of 5% S9 mix/plate
Induced or not induced	Aroclor 1254-induced at 500 mg/kg, 5 days prior to sacrifice
Concentrations tested	1.3, 4.2, 13.0, 43.2, or 130 µg/plate
Statistical Methods	None reported. Criteria for positive response was increase in revertant colonies at least two-fold background at two increasing dose levels.
Remarks for Test Conditions	1-butene was prepared for biological testing by diffusion into ethanol. Ethanol was placed in a gas washing bottle fitted with a cylinder diffuser. 1-butene was bubbled through the solvent for 10 minutes at 0^0C. Samples were transferred to Teflon –capped vials and delivered for Ames testing. Aliquots were removed for GC/FID analysis and comparison with standard samples of undiluted 1-butene. The highest mutagenicity test dose was limited by solubility of 1-butene in ethanol to 130µg/plate. Test sample at 100µl was introduced to a preincubation mixture containing 100µl of log-phase bacteria, 500µl of 5% S9 mix or buffer solution for non-activated tests, and 600 µl of overlay agar per plate which completely filled each vial allowing no headspace. Mixtures were incubated at 37^0C for 10 minutes without shaking. Contents of vials were equally distributed on 3 plates/dose level and incubated at 37^0C for 48 hours. Positive control compounds were sodium azide (TA100), 9-aminoacridine (TA97), 2-nitrofluorene (TA98) for non-activated tests, and 2-aminoanthracene for all S9 assays.
Results	
Genotoxic effects	1-butene did not induce increases in revertant colonies at any dose level up to 130µg/plate in any strain of Salmonella tested with or without metabolic activation Positive controls responded appropriately, inducing 3-17 fold increases over controls –S9 for individual chemical in relevant strain, and 6-20 fold increases +S9 or 8-70 fold increases + hamster S9 with 2-aminoanthracene
Conclusions	
(contractor)	1-butene is not a bacterial mutagen in this test system
Data Quality	
Reliabilities	**2**. Reliable with restrictions. Study performed to develop new methods to deliver ambient air vapors to bacterial test systems. Study was performed according to standard procedures for the Ames assay with analytical characterization of test compounds. GLPs were not cited.
Reference	Claxton, L.D. 1984. Validation of Chemical and Biological Techniques for Evaluation of Vapors in Ambient Air/Mutagenicity Testing of Twelve (12) Vapor-Phase Compounds. EPA Health Research Lab., Research Triangle Park, NC. EPA-600/1-84-005. Contract # 68-02-3170-082
Other	
Last changed	Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vitro

Test Substance	
Test substance	1-butene CAS# 106-98-9, 2-butene 107-01-7 supplied by Tokyo Kasei Co. Ltd.
Method	
Method/guideline followed	New method employs gas sampling bag exposure of 1,3 butadiene and 14 additional gases for in vitro mutagenicity testing
Type	Reverse mutation bacterial
System of testing	Ames bacterial assay with and without metabolic activation and E. coli
GLP	No
Year	1994
Species/Strain	S. typhimurium TA98, TA100, TA1535, TA1537; E. coli WP2 uvrA
Metabolic activation	Yes
Species and cell type	Sprague Dawley rat liver
Quantity	100 µl S9/plate
Induced or not induced	Induced with phenobarbitol and 5,6 benzoflavone
Concentrations tested	500 ml exposure vol./plate, max. 50% gas concentration. Gases diluted with HEPA filtered air.
Statistical Methods	None used
Remarks for Test Conditions	Test substances were collected from cylinders into a 20 liter gas sampling bag. A separate gas bag was filled with a fixed amount of dilution gas (HEPA filtered air). A fixed volume of the test gas was pumped into the dilution bag and mixed. Concentrations were calculated by the volume of both the test gas and the dilution air. Characterizations of undiluted test gases and samples of diluted gases from the mixed gas bag were performed by GC/FID. Standard exposure conditions were: bacterial plates made by agar overlay method using 2 ml top agar/plate, 100 µl S9 or phosphate buffer, 0.1 ml bacteria. Bacterial strains were prepared fresh by preincubating for 10 hours prior to use. When agar overlay was set ,plates were placed separately, upside-down without lids in a plate holder and placed in a 10 liter gas sampling bag. The bag was closed and sealed with adhesive tape and air was evacuated. The bag was then filled with a diluted test butene at an adjusted concentration at a fixed amount per plate (4 plates/dose) and incubated for 24 hours at 37^0C. At termination of exposure, sterile air was pumped in to replace test atmosphere; plates were removed and allowed to stand in a safety cabinet for 30 min to evaporate all residual gas. Lids were replaced on the plates which were incubated for 24 hours at 37^0C.
Results	
Genotoxic effects	1-butene and 2-butene did not induce mutagenic events in any strain in this assay with or without metabolic activation. Only maximum dose was reported (500 ml) and no specific revertant data were supplied for non-mutagenic gases
Conclusions	
(contractor)	1-butene and 2-butene were not mutagenic in this test system employing a gas sampling bag exposure method. Positive results for 1,3-butadiene and 6 other gaseous compounds confirm the acceptability of this method.
Data Quality	
Reliabilities	**2.** Reliable with restrictions Specific data for non-mutagenic gases is limited; control values, dose ranges and revertant data are not reported. Data for positive mutagens are more complete and conform to published results. GLPs were not cited.

Reference	Araki, A., Noguchi, T., Kato, F., and Matsushima, T. (1994) Improved method for mutagenicity testing of gaseous compounds using a gas sampling bag. Mut. Res. 307: 335-344
Other	
Last changed	Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vivo

Test Substance	
Remarks	1-butene, colorless gas with slight aromatic odor. Stability and purity data referred to study sponsor.
Method	
Method/guideline followed	Comparable to standard micronucleus assays, cites Salamone, MF (1983) in Chemical Mutagens vol. 8. Eds. De Serres & Hollaender, Plenum Press NY
Type	Mammalian Bone Marrow Erythrocyte Micronucleus Test
GLP	Yes
Year	1985
Species	Mouse
Strain	Crl:CDR(IRC)Br Swiss
Sex	Male and female; pretest 2M,2F/group: full study 10M, 10F/group & one group of 15M,15F.
Route of administration	Whole body inhalation
Doses/concentration levels	Pretest 1000, 9000, 18,000 ppm; full study 1000, 9000, 22,000 ppm
Exposure period	2 hours/day for 2 days: one group received 22,000 ppm 2 hrs/day for 1 day.
Statistical methods	Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
Remarks for Test Conditions.	1-butene was premixed with ambient air and introduced into inhalation chambers containing groups of mice (10M,10F) at concentrations of 0, 1000, 9000,or 22,000ppm 2 hrs/day for 2 days. One half of each group was killed on day3 and the remainder on day 4 following exposure. One group (15M, 15F) exposed for one day to 22,000 ppm was killed on days 2, 3, 4 after treatment (5/sex/day) Test concentrations were monitored each day by gas chromatography. Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of bone marrow smears were prepared, stained with May-Grunewald/Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all mature erythrocytes(NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.
Results	
Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)	Mice at all doses were unconscious during exposure to 1-butene but recovered when exposure ended. No other clinical signs were observed and no mortality occurred at any dose level. Inhalation of 1-butene by mice did not induce significant changes in micronucleus formation in PCEs or NORMs and did not cause significant changes in the ratio of PCE/NCE ratio of 0.8% in all trated animals compared to 0.9% in negative controls). NOAEL = 22,000 ppm
Conclusions (study authors)	1-butene given by inhalation 2 hrs/day for 2 days to mice had no effect on the frequency of micronucleated erythrocytes in bone marrow. Under these test conditions, 1-butene does not induce chromosome damage.

Data Quality *Reliabilities*	**1.** Reliable without restriction. Study conforms to standard design. GLP have been followed and final QA statement is included in the report.
References	Khan, S.H. Ward, C.O. 1985. Micronucleus test of Gulftene® 4. Unpublished report # 84-2113 by Gulf Life Sciences Center for Gulf Oil Chemicals Co
Other *Last changed*	Rev. 6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Acute Toxicity

Test Substance	2-butene, cis 96.18% pure. Purity determined by Nat'l Bureau of Standards freezing point method.
Method	
Method/guideline followed	No guideline specified, acceptable scientific method
Type (test type)	Acute Effects Evaluation
GLP	No
Year	1950
Species/Strain	Mouse, strain not reported
Sex	not specified
No. of animals per sex per dose	approx 64 mice used to obtain each reported value
Vehicle	oxygen
Route of administration	whole body inhalation
Test Conditions	The purpose of this research study was to compare the anesthetic properties of 20 different highly purified unsaturated hydrocarbons and carbon-oxygen ring compounds in mice (sex/age not reported). Concentrations required for surgical anesthesia and for respiratory arrest were measured. Experiments were carried out in a large stoppered jar equipped with apparatus to introduce known quantities of oxygen (21%) or test compound at 25-27^{0}C under atmospheric pressure. CO2 was absorbed with NaOH. Experiments were limited to concentrations causing anesthesia in 10 min. and were terminated after 20 min. Probit analysis was used to determine conc/effect relationships.
Results	
LC_{50} with confidence limits.	LC50s were not measured. For 2- butene cis, surgical anesthesia occurred at 17.2%, and respiratory arrest at 25.5% giving an anesthetic index of 1.5.
Remarks	
Conclusions (study author)	Results support the concept that narcotic potency increases with molecular weight and degree of unsaturation.
Data Quality Reliability	**2**. Reliable with restrictions. This is not a standard acute toxicity study. It is research study using non-standard methods. Mouse strain and sex were not specified. Methods were appropriate for the purpose.
References	Virtue, R.W. 1950. Anesthetic Effects in Mice and Dogs of Some Unsaturated Hydrocarbons and Carbon Oxygen Ring Compounds. Proc. Soc. Exp. Biol. Med 73:259-262. (See additional acute summaries on other mouse and dog research)
Other	
Last changed	Rev.6/25/2001 (Prepared by a contractor to the Olefins Panel)

Acute Toxicity

Test Substance	2-butene, trans 98.92% pure. Purity determined by Nat'l Bureau of Standards freezing point method.
Method	
Method/guideline followed	No guideline specified, acceptable scientific method
Type (test type)	Acute Effects Evaluation
GLP	No
Year	1950
Species/Strain	Mouse, strain not reported
Sex	not specified
No. of animals per sex per dose	approx 64 mice used to obtain each reported value
Vehicle	oxygen
Route of administration	whole body inhalation
Test Conditions	The purpose of this research study was to compare the anesthetic properties of 20 different highly purified unsaturated hydrocarbons and carbon-oxygen ring compounds in mice (sex/age not reported). Concentrations required for surgical anesthesia and for respiratory arrest were measured. Experiments were carried out in a large stoppered jar equipped with apparatus to introduce known quantities of oxygen (21%) or test compound at 25-27^{0}C under atmospheric pressure. CO2 was absorbed with NaOH. Experiments were limited to concentrations causing anesthesia in 10 min. and were terminated after 20 min. Probit analysis was used to determine conc/effect relationships.
Results	
LC_{50} with confidence limits.	LC50s were not measured. For 2- butene trans, surgical anesthesia occurred at 18.7%, and respiratory arrest at 21.0% giving an anesthetic index of 1.1.
Remarks	
Conclusions (study author)	Results support the concept that narcotic potency increases with molecular weight and degree of unsaturation.
Data Quality Reliability	**2**. Reliable with restrictions. This is not a standard acute toxicity study. It is research study using non-standard methods. Mouse strain and sex were not specified. Methods were appropriate for the purpose.
References	Virtue, R.W. 1950. Anesthetic Effects in Mice and Dogs of Some Unsaturated Hydrocarbons and Carbon Oxygen Ring Compounds. Proc. Soc. Exp. Biol. Med 73:259-262. (See additional acute summaries on other mouse and dog research)
Other *Last changed*	Rev.6/25/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Acute Toxicity

Test Substance	Butene-2 (=95%; 42.4% cis, 55.3% trans), CAS number 107-01-7
Method	
Method/guideline followed	OECD guideline 403 (1981)
Type (test type)	Acute (limit test)
GLP	Yes
Year	1992
Species/Strain	Rat: Wistar [Crl:WI(WU)BR]
Sex	Males and females
No.of animals per sex per dose	5
Vehicle	Filtered air
Route of administration	Inhalation (whole body)
Test Conditions	During exposure, rats were housed individually in wire mesh stainless steel cages within the inhalation chamber (Hazleton Systems Inc, H1000) at a mean temperature of 23.1^0 C and 49% relative humidity. Chamber concentrations of test article were monitored with a total carbon analyzer (FID) calibrated by passing known atmospheres containing test article over the FID. Rats were exposed for 4 hrs to a test article vapor concentration of 23.1 g/m^3 (actual, approx. 10,000 ppm). After exposure, rats were removed from the chambers and returned to their individual living cages for 14 days of observation; the animal room was maintained at 21.5-23^0 C with relative humidity of 38-67% and a 12 hr light/dark cycle. Diet and water were available ad lib. Body weight was measured before study initiation and at post-dose days 7 and 14. Rats were observed for clinical signs during exposure, shortly after, and once daily during the observation period. After the observation period, rats were sacrificed, necropsied, and examined for gross pathological changes.
Results	
LC_{50} with confidence limits.	LOEL not determined NOEL = 23.1 g/m^3 (approximately 10,000 ppm)
Remarks	Restlessness was observed periodically during and after exposure; no clinical signs were seen during the 14 day observation period. Normal growth also occurred during the observation period. No abnormalities were observed at gross necropsy.
Conclusions	
(study author)	From the results of the present study, it was concluded that the 4-hr LC50 value of butene-2 was higher than 23.1g/m^3.
Data Quality	
Reliability	**1.** Reliable without restrictions.
References	Arts, J.H.E. 1992. Acute (4-hour) inhalation toxicity study of butene-2 in rats. Report No. V92.183/352130. TNO Nutrition and Food Research, Zeist, The Netherlands.
Other	
Last changed	5/17/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vitro

Test Substance	
Test substance	2-butene, supplied by Tokyo Kasei Co. Ltd., CAS number 107-01-7
Method	
Method/guideline followed	New method employs gas sampling bag exposure of 1,3 butadiene and 14 additional gases for in vitro mutagenicity testing
Type	Reverse mutation bacterial
System of testing	Ames bacterial assay with and without metabolic activation and E. coli
GLP	No
Year	1994
Species/Strain	S. typhimurium TA98, TA100, TA1535, TA1537; E. coli WP2 uvrA
Metabolic activation	Yes
Species and cell type	Sprague Dawley rat liver
Quantity	100 µl S9 homogenate in 0.5 ml S-9 mix/plate
Induced or not induced	Induced with phenobarbitol and 5,6-benzoflavone (dosage and treatment not specified)
Concentrations tested	500 ml exposure vol./plate, max. 50% gas concentration. Gases diluted with HEPA filtered air.
Statistical Methods	None used
Remarks for Test Conditions	Test substance was collected from a cylinder into a 20 liter gas sampling bag. A separate gas bag was filled with a fixed amount of dilution gas (HEPA filtered air). A fixed volume of the test gas was pumped into the dilution bag and mixed. Concentration was calculated by the volume of both the test gas and the dilution air. Characterization of undiluted test gas and samples of diluted gas from the mixed gas bag were performed by GC/FID. Standard exposure conditions were: bacterial plates made by agar overlay method using 2 ml top agar/plate, 100 µl S9 or phosphate buffer, 0.1 ml bacteria. Bacterial strains were prepared fresh by preincubating for 10 hours prior to use. When agar overlay was set, plates were placed separately, upside-down without lids in a plate holder and placed in a 10 liter gas sampling bag. The bag was closed and sealed with adhesive tape and air was evacuated. The bag was then filled with diluted 2-butene at an adjusted concentration at a fixed amount per plate (4 plates/dose) and incubated for 24 hours at 37^0 C. At termination of exposure, sterile air was pumped in to replace test atmosphere; plates were removed and allowed to stand in a safety cabinet for 30 min to evaporate all residual gas. Lids were replaced on the plates which were incubated for 24 hours at 37^0 C.
Results	
Genotoxic effects	2-butene did not induce mutagenic events in any strain in this assay with or without metabolic activation. Only maximum dose was reported (50% conc.) and no specific revertant data were supplied for non-mutagenic gases
Conclusions	
(contractor)	2-butene was not mutagenic in this test system employing a gas sampling bag exposure method. Positive results for 1,3-butadiene and 7 other gaseous compounds confirm the acceptability of this method.
Data Quality	
Reliabilities	**2.** Reliable with restrictions Specific data for non-mutagenic gases is limited; control values, dose ranges and revertant data are not reported. Data for positive mutagens are more complete and conform to published results

<u>Reference</u>	Araki, A., Noguchi, T., Kato, F., and Matsushima, T. 1994. Improved method for mutagenicity testing of gaseous compounds using a gas sampling bag. Mut. Res. 307: 335-344. (See separate summary for data on 1-butene)
<u>Other</u>	
Last changed	Revised 5/17/2001 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

<u>Test Substance</u>	
Test substance	Butene-2 (42.4% cis, 55.3% trans) from Union Carbide Industrial Gases. Certificate of analysis from supplier. CAS number 107-01-7
<u>Method</u>	
Method/guideline followed	OECD Guideline #471 (1981), Method B14 of Commission Directive 84/449/EEC
Type	Reverse mutation in bacteria
System of testing	Salmonella typhimurium with and without metabolic activation
GLP	Yes
Year	1992
Species/Strain	Salmonella typhimurium TA 1535, TA1537, TA98, TA 100
Metabolic activation	Yes
Species and cell type	Sprague Dawley male rat liver (S9 fraction)
Quantity	10% S9 fraction in S9 mix, (0.05 ml S9 fraction/plate)
Induced or not induced	Aroclor 1254 induced; 500mg/kg single ip injection 5 days before sacrifice.
Concentrations tested	0.0, 10, 20, 40, 60, 80%
Statistical Methods	Dunnett's method of linear regression
Remarks for Test Conditions	A 0.1 ml aliquot of Salmonella, 2.0 ml molten top agar, 0.5 ml S9 mix or 0.5 ml pH 7.4 phosphate buffer were mixed in a test tube and poured on minimal agar plates (3 plates/ conc./± S9 mix). Atmospheres of varying concentrations were generated by mixing Butene-2 with clean dry air, using precalibrated gas flow meters as gas flow indicators. Mixtures passed into 10L stainless steel containers holding Salmonella plates with triple vented lids. Concentrations were selected based on a preliminary range finding test with TA100 ± S9; dose-related reduction in frequency of revertant colonies and reduced growth of background lawn observed at 80, 100%. Containers holding 3 stacks of 8 plates each were flushed with appropriate concentrations of butene-2 for 5 min to allow system to equilibrate; containers were incubated at 37^0 C for 48 hrs and number of revertant colonies counted. Analytical determinations were performed by GC on syringe samples of test atmospheres at representative concentrations. Positive control compounds were: -S9, N-ethyl-N' nitro-N-nitrosoguanidine, 3 µg/plate for TA100, 5 µg/plate for TA1535; 9 amino acridine, 80 µg/plate for TA1537; 4-Nitroquinoline-1-oxide, 0.2 µg/plate for TA98; +S9, 2-aminoanthracene 2 µg/plate for TA1535; benzo(a)pyrene 5 µg/plate for all other strains. Vinyl chloride 50% conc. was gaseous positive control for all strains; negative control was clean dry air. The complete experiment was repeated using fresh bacteria cultures, test material and control solutions. Criteria for positive response were induction of dose-related and statistically significant increases in mutation rate in one or more strain of bacteria ± S9 in both experiments at subtoxic doses.
<u>Results</u>	
Genotoxic effects	Toxicity was exhibited in all strains at 80% butene-2. In experiment 2, slight toxicity also occurred at 60%. No significant increases in number of revertant colonies of any strain of bacteria were observed at any dose concentration ± S9. Controls performed appropriately
<u>Conclusions</u>	
(contractor)	Butene-2 was not mutagenic in the Salmonella typhimurium assay with or without metabolic activation
<u>Data Quality</u>	
Reliabilities	**1.** Reliable without restrictions
<u>Reference</u>	Thompson, P.W. 1992. Butene-2: Reverse mutation assay "Ames test" using Salmonella typhimurium. Proj. #44/812. SafePharm Laboratories, UK, Derby

Other	UK.
Last changed	5/17/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Butene-2 (42.4% cis, 55.3% trans) from Union Carbide Industrial Gases. Certificate of analysis from supplier. CAS number 107-01-7
Method	
Method/guideline followed	OECD Guideline 473 (1981), Method B10 of Commission Directive 84/449/EEC
Type	Chromosome aberrations in mammalian cells.
System of testing	Metaphase analysis in primary blood lymphocyte cultures
GLP	Yes
Year	1992
Species/Strain	Rat – Sprague Dawley (CD-1) males, ages 8-20 wks. from Charles River UK.
Metabolic activation	Yes
Species and cell type	Sprague Dawley male rat liver (S9 fraction)
Quantity	20% S9 fraction in S9 mix, (10% v/v S-9 mix/flask)
Induced or not induced	Aroclor 1254 induced; 500 mg/kg single ip injection 5 days before sacrifice.
Concentrations tested	0.0, 10, 20, 40, 50, 60, 80, 100%
Statistical Methods	Frequency of cells with aberrations (± gaps) and frequency of polyploid cells (duplicate culture data pooled) were compared with concurrent vehicle control using Fisher's Exact Test UKEMS, Statistical Evaluation of Mutagenicity Test Data (1989).
Remarks for Test Conditions	Atmospheres of varying concentrations were generated by mixing Butene-2 with clean dry air, using precalibrated gas flow meters as gas flow indicators. Mixtures passed through culture flasks for sufficient time (time not specified) to allow equilibration of the system. Analytical determinations were performed by GC on syringe samples of test atmospheres at representative concentrations. Blood samples were drawn from male rats; cells were grown in RPMI medium supplemented with 10% fetal calf serum, 25 mM Hepes and antibiotics, at 37^0 C in a humidified atmosphere of 5% CO_2 in air. Duplicate cultures were incubated for 48 hrs, then transferred to tubes, centrifuged and culture medium drawn off and saved. Cells were resuspended in flasks, in fresh culture medium with or without S9 metabolic activation mix and exposed to appropriate concentrations of butene-2 or control materials. Flasks were sealed and shaken to maximize cell exposure for 4 hrs +S9 or 20 hrs -S9. Cells exposed to butene-2 + S9 were resuspended after 4 hrs in original culture medium; one group was harvested at 20 hrs (16 hr recovery), the other at 30 hrs (26 hr recovery) after initiation of treatment; -S9 cultures were harvested after 20 full hrs exposure to butene-2. Positive controls were ethyl methyl sulfonate (500 µg/ml) –S9, cyclophosphamide (4.2 µg/ml) +S9; gaseous control was vinyl chloride (50%) in 20 hr group –S9 and 30 hr group +S9. Negative control was clean, dry air.
Results	
Genotoxic effects	Butene-2 caused hemolysis in +S9 cultures at concentrations of 50% and above. In –S9 cultures, 80 and 100% concentrations caused cultures to turn dark brown but return to normal red color by cell harvest. Butene-2 induced steep dose-related decreases in mitotic indices ± S9; especially toxic to lymphocytes at 80% in +S9 20 hr harvest group. However, butene-2 did not induce significant dose-related increases in frequency of structural chromosome aberrations or polyploid cells at any concentration level at any harvest period ± S9. Control compounds performed appropriately.
Conclusions	
(contractor)	Butene-2 produced no significant increases in frequency of chromosome aberrations either in the presence or absence of a liver enzyme metabolizing system. Butene-2 is not clastogenic to rat lymphocytes in vitro.

Data Quality *Reliabilities*	**1.** Reliable without restrictions
Reference	Wright, N.P. 1992. Butene-2: Metaphase analysis in rat lymphocytes in vitro. Proj. #44/813. SafePharm Laboratories, UK, Derby UK.
Other *Last changed*	5/17/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 2: Low Butadiene C4

Repeated Dose Toxicity

Test Substance	
Remarks	Butene-2 (cis and trans =95%), mol. wt 56.1, from UCAR Specialty Gases, The Netherlands. Certificate of analysis provided by the supplier. CAS number 107-01-7
Method	
Method/guideline followed	OECD guideline 422 (draft 1992, final 1996) Combined repeated dose toxicity and reproductive/developmental toxicity test. Used in SIDS
Test type	Subchronic toxicity
GLP	Yes
Year	1992
Species	Rats
Strain	Wistar (Hsd/Cpd:WU) from Charles River, Sulzfeld, F.R.G.; 13 wks old at study initiation.
Route of administration	Whole body inhalation
Duration of test	39-46 days
Doses/concentration levels	0, 2500, 5000 ppm
Sex	Males and females (12 M, 12 F/group)
Exposure period	Males: 39-46 days; Females: pre-mating, mating through Gestation day 19
Frequency of treatment	6 hr/day, 7 days/wk.
Control group and treatment	12 M, 12 F; filtered air-conditioned air, 6 hr/day, 7 days/wk.
Post exposure observation period	None
Statistical methods	Clinical findings and pathological changes evaluated Fisher's exact probability test. Body wt and food consumption analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.
Test Conditions	Male and female rats (avg. wt. 299.4 g males, 204.0 g females at study initiation) were assigned to one of three groups by computer randomization based on body weight, and uniquely identified by ear tattoo. During the entire exposure period, animals were housed individually in stainless steel cages within modified multitiered Hazleton 1000 inhalation chambers. Temperature range of 20-23^0 C, and relative humidity of 37-80% were monitored continuously using thermo-hygrometers with approximately 10 air changes/hour. Lighting in the animal room and Hazleton chamber was 12 hr light/dark cycle. Animals received food and water ad lib except for ½ hr prior to and during exposure. Animals were exposed to a continuous supply of fresh test atmosphere, passed from a cylinder via a pressure reducer, stainless steel tubing and 2 calibrated mass flow controllers and rotameters to the inlet at the top of the inhalation chamber (2.2 m^3 capacity), where it was diluted with filtered air-conditioned air to appropriate concentration, directed downward to the animal cages, and eventually exhausted out at the bottom of the chamber. Control rats were exposed to filtered air only. Air flow was monitored by an anemometer and recorded three times/exposure day, providing 11-12 air changes/hr. Concentrations of test material were determined with a total carbon analyzer using FID, twice/hr. in each test atmosphere by sampling at locations close to the animal cages. Uniform distribution of butene-2 vapor was verified during preliminary experiments. Nominal concentrations were calculated by mean amount of test material used/hr. divided by mean hourly volume of air passed through the exposure chamber. Top dose level of 5000 ppm was chosen because the estimated body burden was approx. 1000 mg/kg/day, the limit dose for teratology studies in OECD protocol 414. After 2 wks pre-mating exposure, males and females were

	caged together (1:1) until mating had occurred or one week. Mated females were exposed through day 19 of gestation; males and females that did not mate (1 in control group) were exposed until necropsy at the end of the study. However, data from non-pregnant females was not presented. At terminal necropsy, blood was collected from all parental (F0) animals (males and dams) for hematology and clinical chemistry. Organs were excised and weighed (liver, kidney, thymus, lung, testes, epididymides) and 15 organs/tissues processed for microscopic examination: the 6 previously mentioned plus the nose, trachea and larynx, spleen, heart, brain, seminal vesicles, ovaries (after counting corpora lutea), uterus (after counting implantation sites), any abnormal growths or lesions. All organs in the 5000 ppm and control groups were examined by a pathologist.
Results	
NOAEL (NOEL) LOAEL (LOEL) Remarks	NOAEL(systemic) = 2500 ppm (based on body wt changes) Mean actual concentration of butene-2 in test atmospheres was 0, 2476 ± 68ppm (5.7 g/m^3) and 5009 ± 88 ppm (11.5 g/m^3). No mortality or treatment-related clinical signs were observed in parental (F0) animals. Male body wt were comparable in all groups but mean body wt change was statistically significantly lower in the 1st and 4th wk of exposure for 2500 ppm group and in the 1st wk of exposure for 5000 ppm group. Female rats showed statistically significantly decreased mean body wt compared to controls at 14 days from start of exposure in 2500 ppm group and at 7 and 14 days of exposure in 5000 ppm group. During gestation, all body weights were comparable in treated and control groups; on lactation day 1, body wt of 5000 ppm dams was statistically significantly decreased. Body wt changes in dams were comparable to control throughout the study. Food consumption in males was comparable to controls; food consumption by 5000 ppm females was decreased during the first wk of exposure. No other food consumption differences occurred during the study. In hematology data, the total white blood cell count and number of lymphocytes were increased in male rats in both exposure groups compared to concurrent controls, however there was no dose response, values were within historical control range and concurrent control values were low. No changes were observed in % distribution of white blood cells, any red blood cell parameters, or clotting potential. in males or pregnant females of either exposure group. In clinical chemistry data, plasma calcium concentration was slightly decreased in high-dose males but was not considered toxicologically significant since there was no accompanying change in inorganic phosphate levels. No other treatment-related differences were observed. Mean absolute organ wt and relative wt were comparable in all groups. No abnormal, treatment-related macroscopic changes (all groups) or pathological changes (control and 5000 ppm groups) were observed.
Conclusions	
(study authors)	Exposure to Butene-2 at concentrations up to 5000ppm did not induce significant systemic toxicity in male rats exposed for 39-46 days, or in pregnant female rats exposed for 2 weeks pre-mating, through mating and gestation to day 19.
Quality	
Reliabilities	**1.** Reliable without restriction
References	Waalkens-Brendsen, D.H. and Arts, J.H.E. 1992. Combined short term inhalation and reproductive/developmental toxicity screening test with Butene-2 in rats. Proj. #B91-8336 (Study #1410) (see separate summary for reproductive toxicity data)
Other	
Last changed	5/17/2001 (Prepared by a contractor to the Olefins Panel)

Toxicity to Reproduction

Test Substance	
Remarks	Butene-2 (cis and trans =95%), mol. wt 56.1, from UCAR Specialty Gases, The Netherlands. Certificate of analysis provided by the supplier, CAS number 107-01-7
Method	
Method/guideline followed	OECD guideline 422 (draft 1992, final 1996) Combined repeated dose toxicity and reproductive/developmental toxicity test. Used in SIDS.
Test type	Reproductive/Developmental toxicity screening test
GLP	Yes
Year	1992
Species	Rats
Strain	Wistar (Hsd/Cpd:WU) from Charles River, Sulzfeld, F.R.G.; 13 wks old at study initiation.
Route of administration	Whole body inhalation
Duration of test	39-46 days
Concentration levels	0, 2500, 5000 ppm
Sex	Males and females (12 M, 12 F/group)
Exposure period	Males: 39-46 days; Females: pre-mating, mating through Gestation day 19
Frequency of treatment	6 hr/day, 7 days/wk.
Control group and treatment	12 M, 12 F; filtered air-conditioned air, 6 hr/day, 7 days/wk.
Statistical methods	Fisher's exact probability test for parametric data; Kruskal-Wallis analysis of variance followed by Mann-Whitney U-test for non-parametric data. Analysis of variance followed by Dunnet's multiple comparison tests for body weights and food consumption.
Remarks for Test Conditions.	Male and female rats (avg. wt. 299.4 g males, 204.0 g females at study initiation) were assigned to one of three groups by computer randomization based on body weight, and uniquely identified by ear tattoo. During the entire exposure period, animals were housed individually in stainless steel cages within modified multitiered Hazleton 1000 inhalation chambers. Temperature range of 20-23^0 C, and relative humidity of 37-80% were monitored continuously using thermo-hygrometers with approximately 10 air changes/hour. Lighting in the animal room and Hazleton chamber was 12 hr light/dark cycle. Animals received food and water ad lib except for ½ hr prior to and during exposure. Animals were exposed to a continuous supply of fresh test atmosphere, passed from a cylinder via a pressure reducer, stainless steel tubing and 2 calibrated mass flow controllers and rotameters to the inlet at the top of the inhalation chamber (2.2 m^3 capacity), where it was diluted with filtered air-conditioned air to appropriate concentration, directed downward to the animal cages, and eventually exhausted out at the bottom of the chamber. Control rats were exposed to filtered air only. Air flow was monitored by an anemometer and recorded three times/exposure day, providing 11-12 air changes/hr. Concentrations of test material were determined with a total carbon analyzer using FID, twice/hr in each test atmosphere by sampling at locations close to the animal cages. Uniform distribution of butene-2 vapor was verified during preliminary experiments. Nominal concentrations were calculated by mean amount of test material used/hr divided by mean hourly volume of air passed through the exposure chamber. Top dose level of 5000 ppm was chosen because the estimated body burden was approx. 1000 mg/kg/day, the limit dose for teratology studies in OECD protocol 414. After 2 wks pre-mating exposure, males and females were

	caged together (1:1) until mating had occurred or for 1 wk. Mating was verified by a vaginal plug or sperm in a vaginal smear = Gestation day (GD) 0. Pregnant females were exposed through GD19; after which they were removed from the inhalation chambers and housed individually in the animal room, allowed to litter normally and to rear pups to day 4 of lactation, when both dams and pups were killed. Males, and females that did not mate (1 in control group), were housed individually in chambers and exposed until necropsy at the end of the study. Each rat was observed twice a day for reaction to treatment, ill health or mortality. Body wt of males were recorded weekly; body wt of all females were recorded weekly during pre-mating, mated females on GD0, 7, 14, 21, and on lactation days 1, 4. Food consumption was measured weekly for all rats pre-mating and for males after the mating period ended until study termination; for pregnant females, food consumption was recorded weekly during gestation and days 1-4 of lactation. Total litter size and number of pups of each sex, number of stillbirths, grossly malformed pups, if any, and pup body wt were recorded on day 1 and 4 postpartum. Necropsies were performed on stillborns and pups dying during lactation. Macroscopic examinations were performed on these pups and all pups killed on day 4 post-partum, and any abnormalities were recorded. Blood was collected from all parental (F0) animals (males and dams) at terminal necropsy for hematology and clinical chemistry analyses in the subchronic portion of this study. All F0 males and dams were examined macroscopically. Organs were excised and weighed, and tissues processed for microscopic examination. Pregnancies were verified by counting of implantation sites at necropsy; corpora lutea were counted in ovaries prior to fixation. Systemic data from non-pregnant females were not reported.
**Results** NOAEL	 NOAEL(reproductive) = 5000 ppm Mean actual concentration of butene-2 in test atmospheres was 0, 2476 ± 68 ppm (5.7 g/m^3) and 5009 ± 88 ppm (11.5 g/m^3). No mortality or treatment-related clinical signs were observed in parental (F0) animals. Male body wt were comparable in all groups but mean body wt change was statistically significantly lower in the 1st and 4th wk of exposure for 2500 ppm group and in the 1st wk of exposure for 5000 ppm group. Female rats showed statistically significantly decreased mean body wt compared to controls at 14 days from start of exposure in 2500 ppm group and at 7 and 14 days of exposure in 5000 ppm group. During gestation, all body weights were comparable in treated and control groups; on lactation day 1, body wt of 5000 ppm dams was statistically significantly decreased. Body wt changes in dams were comparable to control throughout the study. Food consumption in males was comparable to controls; food consumption by 5000 ppm females was decreased during the first wk of exposure. No other food consumption differences occurred during the study. Mating was successful in 11/12 females in the control group and all females 12/12 in each treated group; precoital times were comparable. Female fecundity index was 73% (8/12), 75% (9/12), 83% (10/12) in control, 2500 ppm and 5000 ppm groups, respectively. Duration of pregnancy was comparable in all groups. One high dose female delivered 1 stillborn pup and 12 live pups; all other dams in all groups delivered live pups. Gestation and live birth indices were approx. 100% in all groups. No treatment-related increase in pre-implantation loss occurred. Post-implantation loss was slightly increased in 5000 ppm group but was within historical control limits and the number of implantation sites in the control group was low. Total number of live births in exposed groups was slightly higher than controls. In the control and 2500 ppm groups, one pup died between days 1 and 4 of lactation, viability index was 97-100%; sex ratio of pups was similar in all groups. Mean body weight of pups was slightly but not statistically significantly lower in 2500 and 5000 ppm groups, which might be explained by the higher number of pups in these groups compared to controls. No treatment related effects

	were noted in pups during lactation or at necropsy.
Conclusions (study authors)	Exposure to butene-2 by inhalation during 2 weeks pre-mating, during mating and the gestation period up to and including day 19 for females, and exposure of males for the entire study (39-46 days) did not induce treatment-related reproductive or developmental toxicity.
Data Quality *Reliabilities*	**1.** Reliable without restriction
References	Waalkens-Brendsen, D.H. and Arts, J.H.E. 1992. Combined short term inhalation and reproductive/developmental toxicity screening test with Butene-2 in rats. Proj. #B91-8336 (Study #1410) (see separate summary for repeat dose toxicity data)
Other *Last changed*	5/17/2001 (Prepared by a contractor to the Olefins Panel)